EEDP-02-4
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# Environmental Effects of Dredging Technical Notes

## UPLAND ANIMAL BIOASSAYS OF DREDGED MATERIAL

PURPOSE: This note follows Technical Note EEDP-02-2 on this subject and provides additional information concerning the concept of using an upland animal as an indicator of bioavailable contaminants in dredged material. The relationship of the WES index species *Eisenia foetida* to field earthworm species likely to colonize an upland dredged material environment is important to the interpretation and prediction of disposal consequences.

This note relates heavy metal uptake by four earthworm field species on eight substrates, which were previously used in WES plant bioassay studies in Europe, to the WES index earthworm *E. foetida*. This is necessary for several reasons. First, the Field Verification Program (FVP) upland animal bioassay must be field verified with a colonizing species and not the laboratory index species. Second, Corps of Engineers District/Division personnel need to know that field species colonizing a site can be related to WES index species. Third, the data presented here will permit the WES plant bioassay data to be related to the animal data in the FVP.

The text of this note was prepared by Dr. E. A. Stafford, Rothamsted Experimental Station, Harpenden, United Kingdom. It is a summary of a report entitled "Comparison of Heavy Metal Uptake by *E. foetida* with That of Other Common Earthworms" prepared by Dr. Stafford in cooperation with Dr. C. A. Edwards, who is now at Ohio State University, through a research contract issued by the European Research Office, US Army Research, Development and Standardization Group, United Kingdom.

BACKGROUND: Animal bioassay test procedures were evaluated, field tested, and verified for dredged material of marine origin under the "Interagency Field Verification of Testing and Predictive Methodologies for Dredged Material Disposal Alternatives" (FVP). The bioassay procedure is not only a relatively simple method for ecological evaluation and environmental assessment of potential upland placement of dredged material, it is a useful postdisposal biomonitoring procedure as well.

The initial laboratory evaluations indicated that the FVP dredged material was toxic to earthworms, and this result was subsequently verified in the field. As the index earthworms did survive in washed FVP Black Rock Harbor (near Bridgeport, Conn.) dredged material, it was suspected that the observed toxicity was directly related to salinity and not to a contaminant.





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Conditions favorable for the colonial establishment of earthworms at the FVP upland field site currently do not exist and probably will occur only when the salinity of the dredged material decreases. At that time, the accumulation of contaminants by the earthworms that form an important part of the terrestrial food web can be related to that predicted by the WES index species. However, the index species functions only as a "white rat" or surrogate in the laboratory and never a prevalent colonial species.

Additional calibration of the upland animal bioassay to biomonitoring procedures, particularly concerning upland habitats on dredged material of marine origin, will be reported in later technical notes.

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## Introduction

Earthworms have great potential for use as bioassay/biomonitor organisms in studies of contaminant uptake and possess many characteristics that make them ideally suited for this purpose (Ma 1982). Studies have demonstrated that native species of earthworms, collected at contaminated sites, can be used to indicate biologically available levels of these contaminants (Helmke et al. 1979, Ireland 1983, Pietz et al. 1984). However, it is the species *Eisenia foetida* (which does not naturally colonize these sites) which has been recommended for use in the laboratory for the ecotoxicological testing of agricultural and industrial chemicals (European Economic Community (EEC) 1984), proposed as a bioassay species for assessing contaminant availability in waste materials, and used to determine the bioavailability of contaminants in dredged material (Marquenie and Simmers 1984).

Correlations between total and DTPA-extractable metal concentrations in contaminated substrates and the concentrations in the tissues of earthworms exposed to these substrates over a 28-day period may be used to establish their potential as biomonitor organisms. Due to differences in physiology and feeding behavior between earthworm species, the use of a surrogate species,

such as *E. foetida*, in studies of contaminant bioavailability needs to be validated by making interspecific comparisons of metal uptake.

## Materials and Methods

### Substrates

Eight substrates consisting of four soils, three dredged materials, and a river sediment were selected which contained elevated levels of at least one of the elements zinc (Zn), copper (Cu), cadmium (Cd), and lead (Pb) as well as a reference soil (Frongoch) with relatively low levels of heavy metals. The soils, dredged material, and sediment were similar to those used previously in bioassays using the WES index plant species. All substrates were air dried and sieved (<2 mm). Their chemical and physical properties are summarized in Table 1.

### Earthworms

Four field species of earthworms were selected for comparison with *E. foetida*. These were *Lumbricus terrestris*, *Allolobophora longa*, *Allolobophora caliginosa*, and *Allolobophora chlorotica*. Earthworms were collected using formalin vermifuge from a substrate of similar metal composition to the reference soil (Frongoch). An initial sample of these earthworms was taken for chemical analysis; a subsample of the *E. foetida* was also analyzed to ensure low background concentrations of heavy metals. Only mature, clitellate earthworms were selected for experimental use in order to ensure comparable age and physiological condition.

### Experimental procedure

Based on European Economic Community test stipulations (EEC 1984) and the results of other research, a stocking density of 1 g (wet weight) of earthworms per 40 g air-dry substrate was used. For the 28-day experimental period, 1,200 g of substrate (air-dry weight) was rewetted to field capacity, and the appropriate number of earthworms (to produce 5 g of tissue at the end of the test period) of each species was added. Each combination of substrate and earthworm species was assayed in triplicate.

After 28-day exposure to the substrates, earthworms were separated from the substrate by hand, weighed, and immediately dried and finely ground for the determination of heavy metal content and acid-insoluble residue. Bioavailability of heavy metals in the substrate was assessed in terms of uptake

Table 1

Physical and Chemical Characteristics of the Soils

| Soil (<2 mm) | Particle Size[1/] 200 µm–2 mm | 50 µm–200 µm | 2 µm–50 µm | <2 µm | Organic Carbon (%C)[2/] | pH (1:2.5) in Water | C.E.C.[3/] | Zn | Cu | Cd | Pb |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Frongoch[4/] | 14 | 6 | 56 | 24 | 4.3 | 6.0 | 20.8 | 116[5/] (3.96)[6/] | 22.9 (5.83) | 0.91 (0.11) | 75.9 (21.9) |
| Ystwyth[7/] | 36 | <1 | 55 | 9 | 2.0 | 6.2 | 7.3 | 790 (184) | 34.7 (9.42) | 3.02 (1.06) | 1,442 (525) |
| Halkyn Mt.[7/] | 6 | 5 | 58 | 31 | 2.5 | 8.1 | 18.4 | 1,383 (332) | 120 (45.2) | 3.40 (2.33) | 945 (195) |
| Shipham[7/] | 50 | 22 | 27 | 1 | 0.7 | 7.6 | 7.7 | 142,674 (637) | 207 (1.50) | 1,616 (25.8) | 22,531 (64.8) |
| Parys Mt.[7/] | 31 | 11 | 45 | 13 | 3.6 | 3.9[8/] | 23.5 | 249 (0.86) | 483 (9.22) | 2.73 (0.02) | 1,443 (1.68) |
| Broekpolder[9/] | 2 | 9 | 57 | 32 | 5.7 | 7.4 | 29.2 | 829 (239) | 154 (50.8) | 10.5 (5.56) | 222 (20.3) |
| Oostabtspolder[9/] | 6 | <1 | 60 | 34 | 4.3 | 7.5 | 22.8 | 446 (126) | 85.9 (29.5) | 9.23 (3.47) | 118 (15.5) |
| Spieringpolder[9/] | 8 | 16 | 47 | 29 | 3.2 | 7.8 | 25.0 | 731 (110) | 88.9 (27.1) | 5.02 (2.11) | 201 (40.4) |
| Neckar River Sediment | 4 | 11 | 55 | 30 | 3.6 | 7.4 | 25.9 | 521 (129) | 202 (79.4) | 27.7 (13.99) | 155 (33.2) |

1/ Particle size separates as a percentage of oven-dry peroxidized substrate <2 mm.

2/ Other percentages based on oven-dry substrate <2 mm.

3/ C.E.C. = cation exchange capacity, measured in milliequivalents/100 g.

4/ Reference soil.

5/ Total ($HNO_3/HClO_4$) metal concentration in substrate.

6/ DTPA-extractable metal concentration in substrate in parentheses.

7/ Soil contaminated by metal mining waste.

8/ Parys Mt. substrate was initially toxic to earthworms, and this toxicity was reduced by addition of lime to raise the pH to 6.0.

9/ Contaminated dredged material.

into the earthworm tissue. It was therefore essential to distinguish between metal concentration in the earthworm tissue and metal concentration due to substrate in the earthworm gut. The acid-insoluble residue content of the earthworm sample was used as an inert marker to calculate the substrate content of the sample, and a correction factor was then applied to eliminate the heavy metal concentration resulting from substrate within the earthworm gut. This method has been described by Stafford and McGrath (1986).

### Chemical analysis

Physical and chemical analysis of the samples was carried out using oven-dried samples of <2-mm particle size. DTPA-extractable metals were measured using a procedure based on the method of Lindsay and Norvell (1978) and modified by Lee et al. (1978). Total metal concentrations were determined after digestion in concentrated nitric and perchloric acids. Concentrations of total and extractable metals were determined by inductively coupled plasma emission spectrometry (ARL® 3400 instrument) with background correction using standards prepared in the appropriate solution. Acid-insoluble residue was determined gravimetrically after digestion in concentrated hydrochloric acid (Stafford and McGrath 1986).

### Statistical methods

Linear regression analyses and associated correlation coefficients were calculated to determine the significance of the relationship between substrate metal levels (total and DTPA-extractable) and metal concentrations in the earthworm tissue, and to compare uptake of metals by *E. foetida* and each of the field species of earthworms.

## Results and Discussion

The percent survival of earthworms over the 28-day period was high, with an overall mean of 92.3 percent (by weight). Metal concentrations present in the earthworm tissue were correlated with metal concentrations (total and DTPA-extractable) in the substrates to which the earthworms were exposed. The linear relationships are plotted in Figure 1, along with correlation coefficients. Data for Zn, Cd, and Pb concentrations showed a skewed distribution and were therefore converted to natural logarithms before carrying out statistical analysis.

Substrate physicochemical variables have been demonstrated to influence

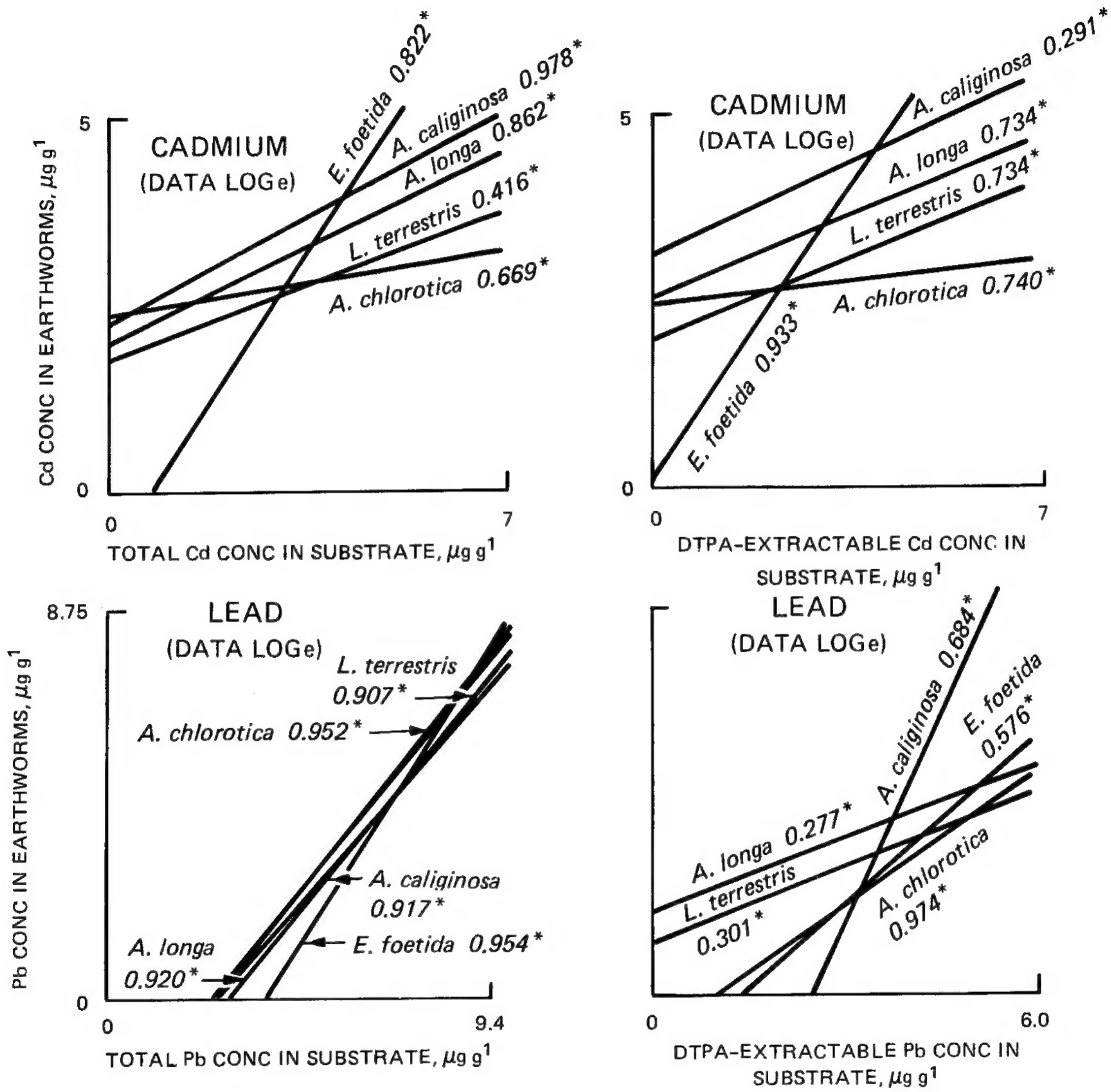


Figure 1. Relationship between metal concentration in earthworms and soil (*mean values significantly correlated within 95-pecent confidence limits) (Continued)

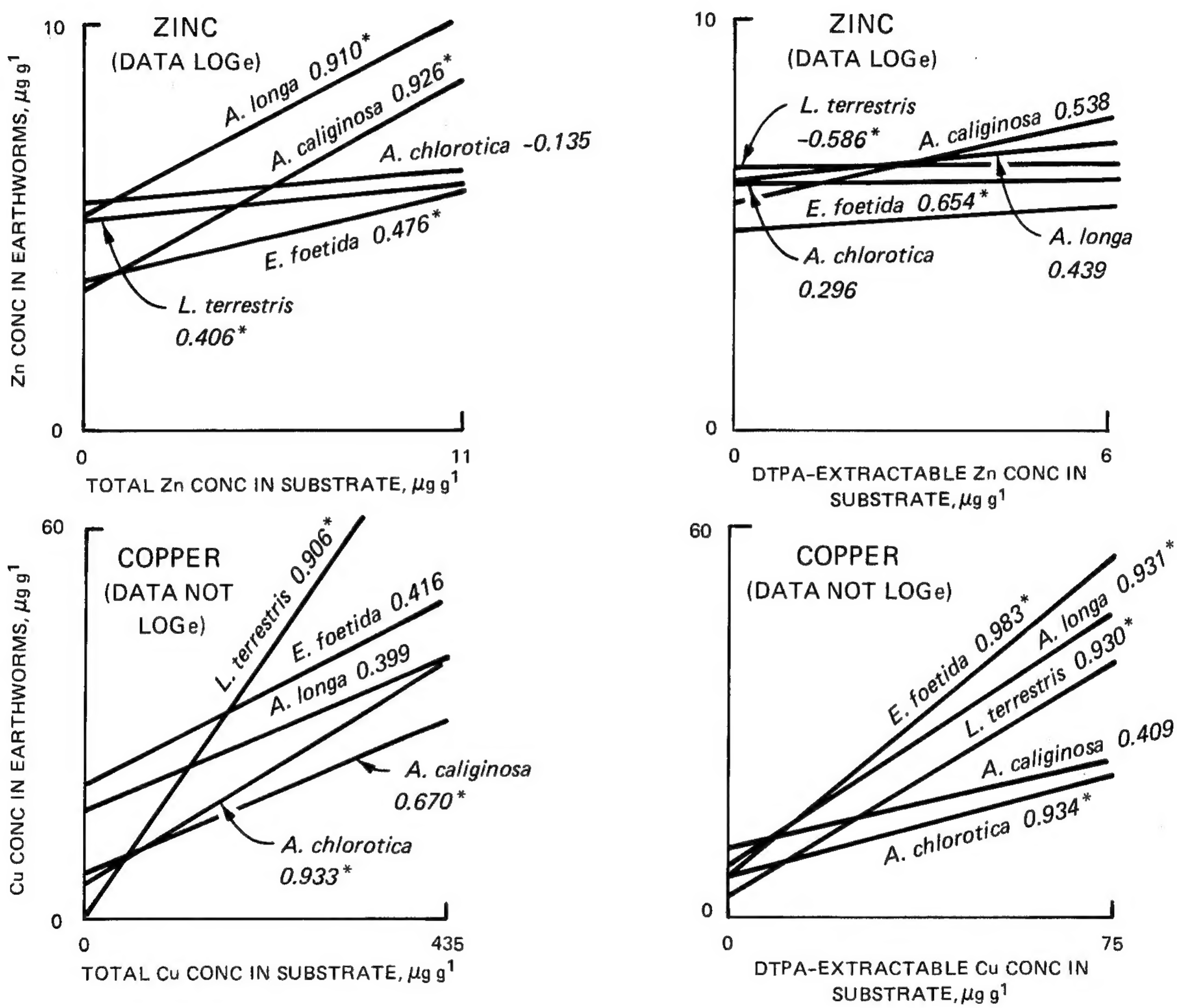


Figure 1. (Concluded)

metal uptake by earthworms (Ma 1982, Ireland 1983); however, despite these additional differences (Table 1), positive linear relationships were observed between earthworm Cu concentrations and DTPA-extractable substrate Cu levels for all species except *A. caliginosa*, between Cd concentrations in most of the earthworm species and total and DTPA-extractable Cd in the substrate, and between Pb concentrations in earthworms and total Pb in the substrate (Figure 1). Cu concentrations in earthworm tissue were more closely correlated with DTPA-extractable Cu in the substrate compared with total Cu in the substrate, while the opposite result emerged for the relationship between earthworm and substrate Pb concentrations. This may have been a result of the chemical extraction procedure used. The DTPA-extraction procedure was developed to assess possible deficiency of Zn, Fe, manganese (Mn), and Cu in the substrate and may provide a good indication of the availability of the elements for which it was developed. However, it is rarely used to assess Pb availability due to the poor results obtained.

Generally, the results obtained from the bioassay suggest that earthworm tissue concentrations of the elements Cu, Cd, and Pb represented a good indication of the bioavailability of these elements in the substrate. In contrast, no consistently significant relationship was evident between Zn concentrations in the earthworm tissue and the substrate. The slope of the line of best fit indicated that Zn concentrations in earthworm tissues increased only slowly with increasing substrate Zn levels (Figure 1). Similar results have previously been reported (Roberts and Johnson 1978, Martin and Coughtrey 1982) and suggest that earthworms may have limited application in assessing the bioavailability of Zn in the substrate.

Metal concentrations present in the earthworm *E. foetida* were also correlated with metal concentrations measured in each of the field species studied. The linear relationships and correlation coefficients are shown in Figure 2. For the majority of combinations, a statistically significant ($p < 0.05$) linear correlation was observed between *E. foetida* and the field species. Although absolute concentrations of metals within the earthworm tissue may differ between species, a close, positive relationship in metal uptake between species, as observed in the present experiment (Figure 2), would suggest that one species can serve to indicate metal availability to the others. As a screening tool, the surrogate species selected for use in laboratory bioassays should represent the most consistency in specific

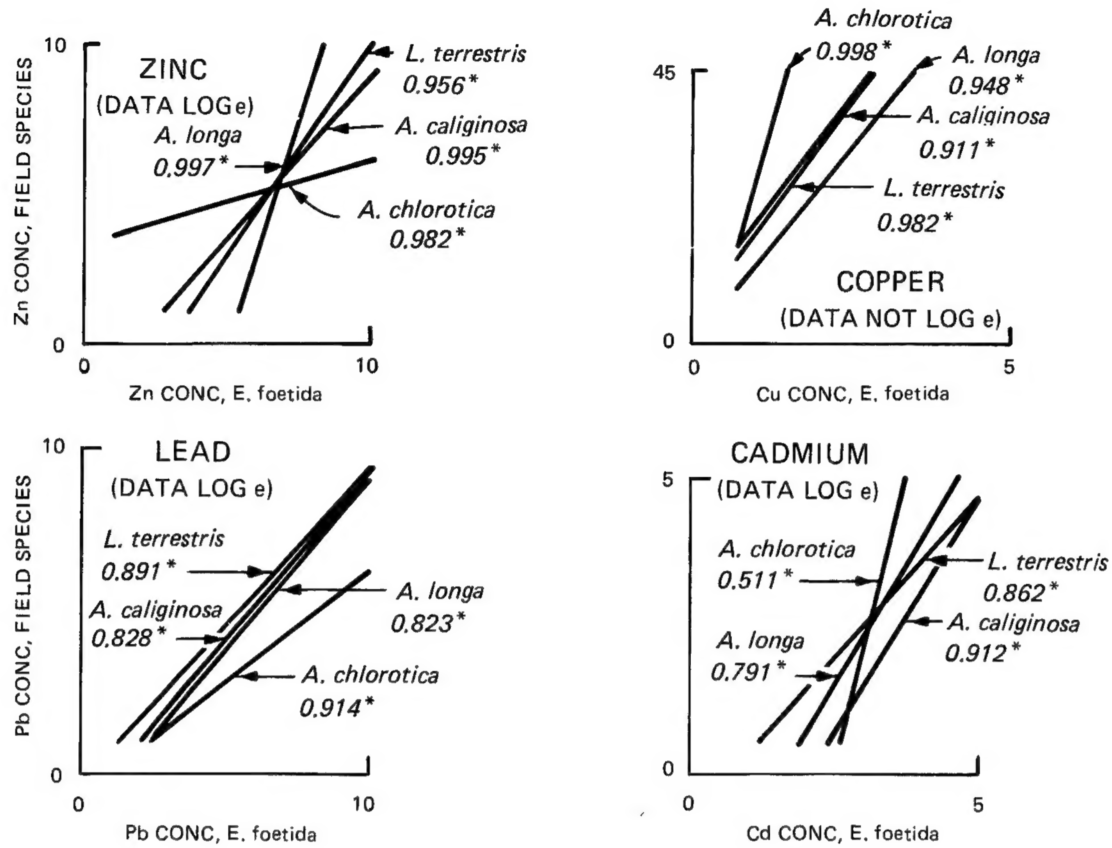


Figure 2. Relationship between metal concentrations in *E. foetida* and field species of earthworm (*mean values significantly correlated within 95 percent confidence limits)

contaminant uptake of the group. Figure 1 generally indicates a high rate of metal uptake by *E. foetida* compared with the field species of earthworm. In addition, Figure 1 shows that there was a consistently significant correlation ($p < 0.05$) between metal concentration in *E. foetida* and increasing metal concentrations in the substrate.

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